bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Proliferation assay in vivo

KS Kishor K Sivaraj
RA Ralf H Adams

Updated date: Nov 27, 2020

An abbreviated version of this protocol was published in eLIFE in Jan 2020

YAP1 and TAZ negatively control bone angiogenesis by limiting hypoxia-inducible factor signaling in endothelial cells

DOI: 10.7554/eLife.50770

Detailed protocol

EdU (5-ethynyl-2'-deoxyuridine) based cell proliferation assay in vivo (Sivaraj et al., 2020)

1. For retina, P6 pups received 100 µg of EdU in 50 µl (2mg/ml) per 3g of body weight intraperitoneally for 2 hr.

2. P6 pups were sacrificed and whole eyes were harvested and fixed in ice cold 4% PFA for 2 hr.

3. After fixation, retinas were dissected and processed as described previously (Pitulescu et al., 2010).

4. After two washes with ice-cold PBS, samples were incubated in blocking buffer for 1 hr on rotating shaker.

5. Next, blocking buffer was replaced by Pblec buffer.

6. Isolectin-B4 (IB4; Vector, Cat#Ab200839) or rabbit monoclonal anti-ERG (Abcam, Cat# ab22552) were diluted in Pblec buffer and each retina was incubated in 100 µl of solution overnight at 4°C.

7. Next, samples were washed five times in incubation buffer (diluted blocking buffer 1:1 in PBS) and incubated with the appropriate Alexa Fluor488, 546 and 594-conjugated secondary antibodies for 2 hr at room temperature.

8. Retinas were washed three times with ice-cold PBS.

9. Retinas were incubated with **Click-it EdU stock solutions** for 30 minutes at room temperature, protected from light.

10. Retinas were washed three-five times with ice-cold blocking buffer.

11. Later, retinas were incubated with DAPI (4′,6-diamidino-2-phenylindole) (1 µg/ml) in blocking buffer for 10 minutes at room temperature, protected from light.

12. Finally, retinas were washed three- five times with ice-cold PBS and mounted under a stereomicroscope.

Solutions:

1. **EdU** (5-ethynyl-2'-deoxyuridine) 1mg/ml (i.e. 2mg EdU, dissolve in 100ul DMSO by vortexing, add 900ul of PBS to a final volume of 1ml).

2. **Blocking buffer** (1%BSA, 1% Triton X-100, 3% heat inactivated donkey serum in PBS).

3. **Pblec buffer** (1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1 mM $MnCl_2$, 0.1% Triton X-100 in PBS).

4. **Click-it EdU stock solutions (f**or 100ul of staining solution mix):

10x Click-it EdU reaction buffer	10 µl
CuSO4	4 µl
Alexa Fluor Azide 647	0.25 µl
10x Click/it EdU buffer additive	10 µl
water	75.75 µl
Click-it EdU stock solutions	100 µl

References

1. Pitulescu, M.E., Schmidt, I., Benedito, R., and Adams, R.H. (2010). Inducible gene targeting in the neonatal vasculature and analysis of retinal angiogenesis in mice. Nature protocols *5*, 1518-1534.
2. Sivaraj, K.K., Dharmalingam, B., Mohanakrishnan, V., Jeong, H.W., Kato, K., Schroder, S., Adams, S., Koh, G.Y., and Adams, R.H. (2020). YAP1 and TAZ negatively control bone angiogenesis by limiting hypoxia-inducible factor signaling in endothelial cells. eLife *9*.

Related files